6.

13. (Amended) The method of claim 1 wherein said agent is a glycoprotein.

7.

14. (Amended) The method of claim 1 wherein said agent is a nucleophile.

## REMARKS

Reconsideration of the present application in view of the above amendments and the following remarks is respectfully requested. Claims 1-14 are pending. Claims 1-14 have been amended to more specifically define the subject matter of the claimed invention. Support for amendments to claim 1 may be found at lines 15-17, page 1; line 25, page 1 to line 2, page 2; and lines 12-13, page 6. In addition, the specification has been amended to eliminate informalities associated with the use of trademark SYBRGREEN®, to replace the word "destroying" with the word "destructive," to provide full names for certain abbreviation, and to clarify The Brief Description Of The Drawings. As discussed in detailed below, support for the amendments to The Brief Description Of The Drawings may be found in the examples of the present application. No new matter has been added.

### Objections to Specification

The specification stands objected to because of certain alleged informalities. More specifically, the Action asserts that the term "destroying" is misused as an adjective. In addition, the Action states that it is unclear to what the phrase "PDAR GAPDH" on page 14 refers. The Action also requests that the trademark SYBRGREEN® be capitalized and accompanied by its generic terminology. The Action further asserts that several errors exist concerning the figures and The Brief Description Of The Drawings.

Applicants respectfully traverse the objection related to the use of the term "destroying." Applicants believe that this word may be used as an adjective to mean "destructive." Nevertheless, to facilitate allowance and without acquiescing to the assertions in

the Action, Applicants have amended the specification to replace "destroying" with "destructive."

Regarding the phrase "PDAR GAPDH," Applicants submit that "PDAR" is the abbreviation for pre-developed assay reagents, and "GAPDH" is the abbreviation for glyceraldehyde-3-phosphate dehydrogenase.

As to the rejection related to the use of the trademark SYBRGREEN®, Applicants have amended the specification to capitalize this trademark and to indicate that SYBRGREEN® dye is a cyanine dye.

The Action notes that in the brief description of Figures 4-6, Applicants claim that multiple samples were tested, yet there are no error bars in the figure. Applicants have amended the brief description of these figures to indicate that the data presented are the average of multiple analyses.

The Action also notes that in the brief description of Figures 5 and 6, there is no indication of what (A) represents. Applicants have amended the brief description of these figures to correct the above informalities. Support for these amendments may be found in Example 5.

The Action notes that in the brief description of Figure 7, there is no indication of what the different lanes represent. Applicants have amended the brief description of this figure to correct the above informality. Support for these amendments may be found in Example 6.

The Action notes that in the brief description of Figure 14, there is no indication of what the different bars represent. Applicants have amended the brief description of this figures to correct the above informality. Support for these amendments may be found in Example 13.

In view of the above remarks, Applicants submit that this ground of objections to the specification has been overcome. Withdrawal of these objections is respectfully requested.

Request for Drawing Change

The Action states that the data shown in Figure 3 seems contradictory to the inventors' conclusion. Applicants hereby submit a Request for Drawing Change, along with one sheet of formal drawings reflecting the change, for approval by the Examiner, in order to correct an error in Figure 3. Support for the change in Figure 3 may be found in Example 3.

In view of the above request, Applicants submit that this ground of objection has been overcome. Withdrawal of this objection is respectfully requested.

Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 1-14 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. More specifically, the Action asserts that claims 1 and 2 do not recite a step that refers back to the preambles of the claims. The Action further claims that claim 11 is indefinite for using the trademark SYBRGREEN®.

As indicated above, Applicants have amended claim 1. The text of amended claim 1 now refers back to its preamble. Regarding the use of the above-noted trademark, Applicants have amended claim 11 to replace the trademark with its generic terminology.

In view of the above remarks, Applicants submit that the ground of rejection under 35 U.S.C. § 112, second paragraph, has been overcome. Withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. § 102

Claims 1, 3-8, 10 and 12-14 stand rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Lader (Published PCT Application WO 00/06780). In addition, claims 2, 5-8, 10 and 12-14 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by the same reference. Claims 1-8, 10 and 12-14 also stand rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by Lader (U.S. Pat. No. 6,204,375).

More specifically, the Action asserts that the cited references both disclose a method of RNA preservation using concentrations of ammonium sulfate that fall within the ranges as recited in claims of the present application. In addition, the Action asserts that these references also disclose a preferred method that results in RNA isolation. The Action further claims that because RNA binding agents, polyamines, a cationic detergent, actinomycin, charged polysaccharides, glycoproteins and nucleophiles are all present in cell lysates, the cited references also anticipate claims 5-8, 10 and 12-14.

To facilitate allowance and without acquiescing to the above assertions in the Action, Applicants have amended claim 1 to more clearly define the claimed subject matter. Amended claim 1 now recites a method to neutralize the inhibitory or destructive effect of an agent that binds or cleaves to RNA molecules, comprising adding ammonium sulfate to a composition that comprises the agent and RNA molecules isolated from a natural source or artificially synthesized. Such a method is not disclosed in either of the cited references. More specifically, these references fail to disclose the step of adding ammonium sulfate to a composition containing RNA molecules that have already been *isolated* or are *artificially synthesized.* Instead, these reference are related to preserving RNA in samples by the use of ammonium sulfate prior to RNA isolation or purification. Because a prior art reference is anticipatory only if it teaches every element of a claimed invention (*Constant v. Advanced Micro-Devices, Inc.*, 848 F.2d 1560, 1570 (Fed. Cir. 1988)), Applicants respectfully submit that neither of the cited references anticipates amended claim 1.

In view of the above remarks, Applicants respectfully submit that the above grounds of rejections under 35 U.S.C. § 102 has been overcome. Withdrawal of these rejections are respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The first of the attached page is captioned "**Version With Markings to Show Changes Made.**" Also enclosed is a copy of Limited Recognition Under 37 CFR § 10.9(b).

On the basis of the above amendments and remarks, reconsideration of the application and its allowance are respectfully requested. Should the Examiner have any additional questions, he is respectfully encouraged to contact the undersigned attorney at (206) 622-4900.

Respectfully submitted,

Christian Korfhage et al.

SEED Intellectual Property Law Group PLLC

Qing Lin, Ph.D.
(See Limited Recognition)

QXL:jab

Enclosures:
1 Sheet of Drawings With Correction in Red
1 Page Formal Drawings (Figs. 3 and 4)
Copy of Limited Recognition Under 37 CFR § 10.9(b).

701 Fifth Avenue, Suite 6300
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Fax: (206) 682-6031

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning at line 7, page 1 has been amended as follows:

It is well known that many molecules such as proteins, spermine, spermidine, cationic detergents, ethidium bromide, ~~SybrGreen™~~ SYBRGREEN® (cyanine) dye, actinomycin, etc. are able to bind to and to inhibit the function and analysis of RNA. The binding mode of most inhibitory molecules to RNA is ionic, stabilized by hydrophilic or lipophilic interaction. In many cases, the interaction between inhibitory molecules and RNA is very strong so that very harsh conditions (*e.g.*, denaturating agents, chaotropic agents, detergents, phenol etc.) are needed to diminish the interaction between RNA and inhibitory molecules. In some cases, even harsh conditions do not stop the interaction. In other cases, the harsh conditions interfere with downstream applications of the RNA. Accordingly, a method is needed which neutralizes or mitigates the interaction of inhibitory molecules to RNA, but does not interfere with the function and analysis of RNA.

The paragraph beginning at line 24, page 1 has been amended as follows:

The invention describes the addition of $(NH_4)_2SO_4$ to an environment containing RNA. The final concentration is below 20 g/100 ml (1.51 M). The addition of $(NH_4)_2SO_4$ to the environment neutralizes the inhibitory effects of agents that bind to or cleave RNA. Such agents include cationic detergents (*e.g.*, CATRIMOX and cetyltrimethylammonium bromide (CTAB). *See, e.g.*, European Patent Application EP 1031626 A1), proteins, ethidium bromide, SYBRGREEN® ~~SybrGreen™~~ dye, polyamines (*e.g.*, spermine, spermidine, putresceine etc.), charged polysaccharides, glycoproteins, nucleophiles, bases etc. In the presence of $(NH_4)_2SO_4$, the inhibitory or cleaving properties of agents that bind to RNA is reduced or eliminated.

The paragraph beginning at line 23, page 2 has been amended as follows:

In any of the aforesaid aspects, the composition may further comprise a contaminant selected from RNA binding agents. In any of the aforesaid aspects, the composition may further comprise a polyamine as a contaminant, where spermine, spermidine, and

putresceine are exemplary polyamine contaminants. In any of the aforesaid aspects, the composition may further comprise a cationic detergent as a contaminant. In any of the aforesaid aspects, the composition may further comprise a nucleic acid dye as a contaminant, where ethidium bromide and SYBRGREEN® ~~SybrGreen™~~ dye are exemplary nucleic acid dye contaminants. In any of the aforesaid aspects, the composition may further comprise actinomycin as a contaminant. In any of the aforesaid aspects, the composition may further comprise a charged polysaccharide as a contaminant. In any of the aforesaid aspects, the composition may further comprise glycoprotein as a contaminant. In any of the aforesaid aspects, the composition may further comprise a nucleophile as a contaminant. In additional aspects, the present invention provides that the composition to which ammonium sulfate is added may contain any two or more of the specifically enumerated contaminants, *i.e.,* any two or more (*e.g.,* three, four) of RNA binding agent, polyamine, cationic detergent, nucleic acid dye, actinomycin, charged polysaccharide, glycoprotein, and nucleophile.

The paragraph beginning at line 21, page 3 has been amended as follows:

Figure 4 shows improvement in the performance of TaqMan RT-PCR by the addition of ammonium sulfate when cationic detergents were present in the reaction mixture. The threshold-cycle of each bar reflects the average of six independent values.

The paragraph beginning at line 24, page 3 has been amended as follows:

Figure 5 shows stability of the neutralization effect during time. RNA prepared in the absence of any cationic detergent with elution in water ~~by classical method~~ (C), ~~or by~~ in the presence of a cationic detergent with elution in water (B), or in the presence of a cationic detergent with elution in 10 ~~5~~ mM $(NH_4)_2SO_4$ (A) was used in TaqMan RT-PCR reactions for amplifying GAPDH (glyceraldehydes-3-phosphate dehydrogenase). Each bar in ~~T~~this figure ~~summarizes~~ represents the average of the analyses of three independent blood donors.

The paragraph beginning at line 1, page 4 has been amended as follows:

Figure 6 shows stability of the neutralization effect during time. RNA prepared in the absence of any cationic detergent with elution in water ~~by classical method~~ (C), in the

presence of ~~or by~~ a cationic detergent with elution in water (B), or in the presence of a cationic detergent with elution in ~~5~~ 10 mM $(NH_4)_2SO_4$ (A) was used in TaqMan RT-PCR reactions for amplifying TNFalpha (Tumor Necrosis Factor alpha). Each bar in ~~T~~this figure ~~summarizes~~ represents the average of the analyses of three independent blood donors.

The paragraph beginning at line 5, page 4 has been amended as follows:

Figure 7 shows inhibitory effects of spermine during reverse transcription reactions. The concentrations of spermine in lanes 1-5 are: 0 mM, 0.125 mM, 0.25 mM, 0.5 mM, and 1 mM, respectively.

The paragraph beginning at line 11, page 4 has been amended as follows:

Figure 9 shows inhibitory effects of SYBRGREEN® ~~SybrGreen™~~ dye during reverse transcription reaction. Final concentrations of SYBRGREEN® ~~SybrGreen™~~ dye in the reverse transcription reaction mixture are indicated. PCR was performed to quantify cDNA synthesis.

The paragraph beginning at line 14, page 4 has been amended as follows:

Figure 10 shows electrophoretic analysis of RT-PCR products from an RNA sample that contains SYBRGREEN® ~~SybrGreen™~~ dye, but no ammonium sulfate (lanes 2), an RNA sample that contains both SYBRGREEN® ~~SybrGreen™~~ dye and ammonium sulfate (lanes 1), and an RNA sample that contains neither SYBRGREEN® ~~SybrGreen™~~ dye nor ammonium sulfate as a control (Lane 3).

The paragraph beginning at line 24, page 4 has been amended as follows:

Figure 14 shows relative activities of a reverse transcriptase using RNAs dissolved in solutions of different $(NH_4)_2SO_4$ concentrations as templates. The bars with three different shades represent results from three different lots of reverse transcriptases.

The paragraph beginning at line 4, page 5 has been amended as follows:

Ribonucleic acid (RNA) is a substance synthesized biologically and synthetically. RNA serves many functions as information molecule, reaction substrate, reaction catalyst, recognition element, structural element, etc. For most analysis methods and functions concerning RNA, the purity of RNA is important. For instance, other molecules present in an RNA sample, or in a reaction mixture ~~that~~ in which RNA molecules participate, may inhibit the analysis or function of the RNA molecules or destroy the structure of the RNA molecules. Thus, it is important to reduce or eliminate the inhibitory or ~~destroying~~ destructive effects of such molecules. Moreover, stable secondary structures of RNA may also interfere with RNA function or analysis. The present invention features the addition of ammonium sulfate to an RNA solution to eliminate or reduce the inhibitory or ~~destroying~~ destructive effects of certain other molecules (*e.g.*, those that bind to the RNA) in the solution that interferes with the use of RNA as information molecule, reaction substrate, reaction catalyst, recognition element, structural element etc. Furthermore, $(NH_4)_2SO_4$ solves secondary structures of RNA to make RNA more accessible to reactions and analysis

The paragraph beginning at line 19, page 5 has been amended as follows:

The present invention is directed to the addition of $(NH_4)_2SO_4$ to a composition containing RNA. In one aspect, the final concentration of the $(NH_4)_2SO_4$ is below 20 g/100 mL (1.51 M). The addition of $(NH_4)_2SO_4$ to the environment neutralizes the inhibitory effects of agents that interferes with RNA function and/or analysis, such as those that bind to, or cleave, the RNA. Such agents include cationic detergents (*e.g.*, CATRIMOX and cetyltrimethylammonium bromide (CTAB). *See, e.g.*, European Patent Application EP 1031626 A1), proteins, ethidium bromide, SYBRGREEN® ~~SybrGreen™~~ dye, polyamines (*e.g.*, spermine, spermidine, putresceine etc.), charged polysaccharides, glycoproteins, nucleophiles, bases, etc.

The paragraph beginning at line 14, page 11 has been amended as follows:

RNA is a substance synthesized biologically and synthetically. RNA serves many functions as information molecule, reaction substrate, reaction catalyst, recognition element, structural element, etc. For most analysis methods and functions concerning RNA, the purity of RNA is important. For instance, other molecules present in an RNA sample, or in a reaction



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mixture that RNA molecules participate, may inhibit the analysis or function of the RNA molecules or destroy the structure of the RNA molecules. Thus, it is important to reduce or eliminate the inhibitory or ~~destroying~~ destructive effects of such molecules. Moreover, stable secondary structures of RNA may also interfere with RNA function or analysis. The present invention features the addition of ammonium sulfate to an RNA solution to eliminate or reduce the inhibitory or ~~destroying~~ destructive effects of certain other molecules (*e.g.*, those that bind to the RNA) in the solution that interferes with the use of RNA as information molecule, reaction substrate, reaction catalyst, recognition element, structural element, etc. Furthermore $(NH_4)_2SO_4$ solves secondary structures of RNA to make RNA more accessible to reactions and analysis.

The paragraph beginning at line 1, page 12 has been amended as follows:

The present invention is directed to the addition of $(NH_4)_2SO_4$ to a composition containing RNA. In one aspect, the final concentration of the $(NH_4)_2SO_4$ is below 20 g/100 mL (1.51 M). The addition of $(NH_4)_2SO_4$ to the environment neutralizes the inhibitory effects of agents that interferes with RNA function and/or analysis, such as those that bind to, or cleave, the RNA. Such agents include cationic detergents (*e.g.*, CATRIMOX and cetyltrimethylammonium bromide (CTAB). *See, e.g.*, European Patent Application EP 1031626 A1), proteins, ethidium bromide, SYBRGREEN® ~~SybrGreen™~~ dye, polyamines (*e.g.*, spermine, spermidine, putresceine etc.), charged polysaccharides, glycoproteins, nucleophiles, bases, etc.

The paragraph beginning at line 22, page 14 has been amended as follows:

Human blood RNA was prepared with a cationic detergent or with a classical method in the absence of any cationic detergent. The RNA was eluted with water or with 10 mM $(NH_4)_2SO_4$. The eluate containing 10 mM $(NH_4)_2SO_4$ was denaturated at 65°C for 5 minutes and cooled on ice. An aliquot of each eluate was transferred to a single-tube TaqMan RT-PCR mixture to amplify a GAPDH fragment. Ingredients of the above reaction were provided by Applied Biosystem (PDAR (Pre-Developed Assay Reagents) GAPDH).

Example 8 at page 17 has been amended as follows:

## EXAMPLE 8

### SYBRGREEN® ~~SYBRGREEN™~~ DYE INHIBITS RT-PCR REACTIONS

This example shows that SYBRGREEN® ~~SybrGreen™~~ dye inhibits RT-PCR reactions. As shown in Figure 9, SYBRGREEN® ~~SybrGreen™~~ dye at a final concentration of 100x resulted in a total loss of cDNA synthesis.

Experimental set-up:

Reverse transcription reactions containing 0x, 0.001x, 0.01x, 0.1x, 1x, 10x and 100x SYBRGREEN® ~~SybrGreen™~~ dye were performed. In order to quantify the generated cDNA after RT-reaction, 2 µl of the RT reaction was transferred to a 20 µl PCR mixture. PCR products were analyzed by gel-electrophoresis.

Example 9 at pages 17 and 18 has been amended as follows:

## EXAMPLE 9

### $(NH_4)_2SO_4$ MITIGATES THE INHIBITORY EFFECTS OF SYBRGREEN® ~~SYBRGREEN™~~ DYE ON RT-PCR

Reverse transcriptase is not able to displace SYBRGREEN® ~~SybrGreen™~~ dye binding to RNA. Thus, RNA is masked and cannot be analyzed quantitatively. Due to the binding of SYBRGREEN® ~~SybrGreen™~~ dye to RNA, only signal with very low intensities were obtained during rRT-PCR. A denaturation step (5 minutes at 65°C, shock cool on ice) of RNA alone did not solve the complex of RNA and SYBRGREEN® ~~SybrGreen™~~ dye. The addition of $(NH_4)_2SO_4$ to the final concentration of 5 mM followed by denaturation of the sample for 5 minutes at 65°C with shock cool on ice significantly increased the RT-PCR signal (Figure 10).

Experimental set-up:

Total RNA containing SYBRGREEN® ~~SybrGreen™~~ dye was dissolved in 2 µl water (lanes 2) or in 2 µl of a 5 mM $(NH_4)_2SO_4$ solution (lanes 1). In lanes 3, total RNA without SYBRGREEN® ~~SybrGreen™~~ dye was dissolved in 2 µl of a 5 mM $(NH_4)_2SO_4$ solution. The



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solution was denaturated at 65°C for 5 minutes and cooled on ice. The whole solution was transferred to a 20 µl RT reaction mixture and the RT reaction was performed at 37°C. After the RT reaction was finished, 2 µl of the RT reaction mixture was transferred to a 20 µl PCR mixture. The resulting PCR products were analyzed by gel-electrophoresis.

In the Claims:

Claims 1-14 have been amended as follows:

1. (Amended) A method ~~of RNA purification~~ to neutralize the inhibitory or destructive effect of an agent on the function or analysis of RNA isolated from a natural source or artificially synthesized, wherein the agent binds to, or cleaves, said RNA, comprising adding ammonium sulfate to a composition comprising said RNA and said agent, where the final concentration of ammonium sulfate in the composition is below 20 g / 100 mL, and whereby the inhibitory or destructive effect of said agent is neutralized.

2. (Amended) The ~~A~~ method of ~~RNA purification~~ claim 1, ~~comprising adding ammonium sulfate to a composition comprising RNA,~~ where the final concentration of ammonium sulfate in the composition is about 1-64 mM.

3. (Amended) The method of ~~RNA purification~~ according to claim 2, where the final concentration of ammonium sulfate in the composition is about 5-32 mM.

4. (Amended) The method of ~~RNA purification~~ according to claim 2, where the final concentration of ammonium sulfate in the composition is about 10 mM.

5. (Amended) The method of claims 1 ~~or 2~~ wherein ~~the composition further comprises a contaminant selected from RNA binding agents~~ said agent binds to said RNA.



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6. (Amended) The method of claims 1 ~~or 2~~ wherein ~~the composition further comprises~~ said agent is a polyamine ~~as a contaminant~~.

7. (Amended) The method of claims ~~6~~ 1 ~~or 2~~ wherein ~~the composition further comprises a~~ said polyamine is ~~contaminant~~ selected from spermine, spermidine, and putresceine.

8. (Amended) The method of claims 1 ~~or 2~~ wherein ~~the composition further comprises~~ said agent is a cationic detergent ~~as a contaminant~~.

9. (Amended) The method of claims 1 ~~or 2~~ wherein ~~the composition further comprises~~ said agent is a nucleic acid dye ~~as a contaminant~~.

10. (Amended) The method of claims 1 ~~or 2~~ wherein ~~the composition further~~ said agent is ~~comprises~~ actinomycin ~~as a contaminant~~.

11. (Amended) The method of claims ~~9~~ 1 ~~or 2~~ wherein the ~~composition further comprises a nucleic acid dye as a contaminant and the~~ nucleic acid dye is ethidium bromide or ~~SybrGreen™~~ cyanine dye.

12. (Amended) The method of claims 1 ~~or 2~~ wherein ~~the composition further comprises~~ said agent is a charged polysaccharide ~~as a contaminant~~.

13. (Amended) The method of claims 1 ~~or 2~~ wherein ~~the composition further comprises~~ said agent is a glycoprotein ~~as a contaminant~~.

14. (Amended) The method of claims 1 ~~or 2~~ wherein ~~the composition further comprises~~ said agent is a nucleophile ~~as a contaminant~~.



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Factor of increase in performance of reverse transcription with inhibited RNA in $(NH_4)_2SO_4$-solution vs. non-inhibited RNA in $(NH_4)_2SO_4$-Solution

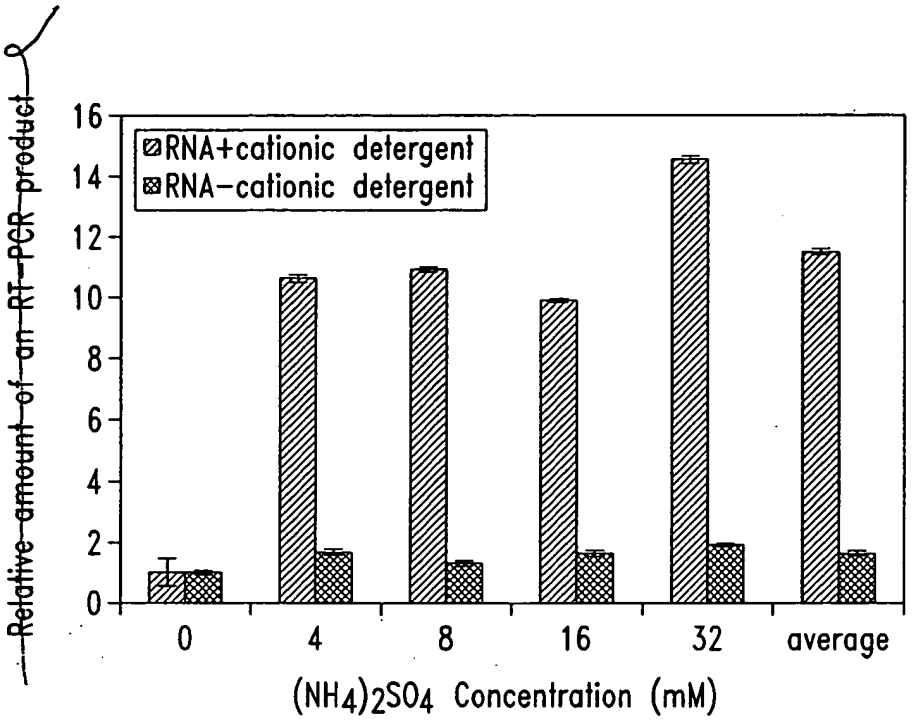
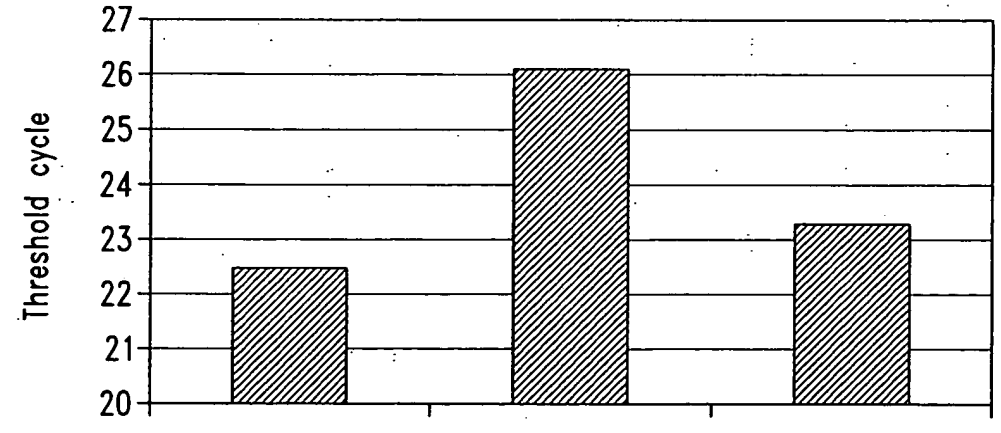



*Fig. 3*




| Cationic detergent | + | + | − |
|---|---|---|---|
| $(NH_4)_2SO_4$ (mM) | 10 | 0 | 0 |

*Fig. 4*